Ultralow Oxygen Treatment for Postharvest Control of Western Flower Thrips on Broccoli

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ABSTRACT Laboratory studies were conducted to develop ultralow oxygen (ULO) treatment for controlling western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), on broccoli at a low temperature of 1°C. Complete control was achieved in 5 d at 0.003% oxygen. Oxygen level affected efficacy of ULO treatment. At a higher oxygen level of 0.03%, 6-d treatment killed ≈85% of thrips, and 10-d treatment killed all thrips. The 5-d ULO treatment with 0.003% oxygen was successfully tested on iced commercial broccoli of several cultivars without any noticeable negative effects on shelf-life and postharvest quality. The ULO treatment provided a safe and effective alternative to methyl bromide fumigation for postharvest control of western flower thrips on exported broccoli for industrial development.

KEY WORDS controlled atmosphere, ultralow oxygen, quarantine treatment, western flower thrips, postharvest quality

Postharvest insect control is critical for international trade of fresh commodities, such as fruits and vegetables, that harbor quarantined pests (Mitcham 2001). Chemical fumigation often results in reduced product quality and shelf-life in addition to high costs and environmental damage. Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a common pest on broccoli, *Brassica oleracea* (L.), in the United States, but it is quarantined in Taiwan, an important export market of U.S. broccoli. The current quarantine treatment with methyl bromide fumigation for thrips control is harmful to the environment, because methyl bromide destroys atmospheric ozone, and it is also likely to become very costly in the future as methyl bromide production is being gradually phased out globally. Broccoli is often shipped with ice to maintain optimal quality. Methyl bromide fumigation is typically conducted at ambient temperature and is not compatible with iced shipping of broccoli due to melting of ice. An effective, safe, and environmental friendly treatment for thrips control on broccoli would promote export of U.S. broccoli to overseas markets and benefit U.S. agriculture as well as the environment.

Controlled atmospheres (CA) have been studied for postharvest pest control on fresh commodities, including broccoli (Carpenter and Potter 1994, Mitcham et al. 2001, Liu 2003). Typical CA treatments are to store commodities under an atmosphere with controlled low levels of oxygen, high levels of CO_2, or a combination for specified periods at certain temperatures to disinfest the commodities of quarantined pests. Most previous studies on postharvest control of thrips on broccoli with CA used CO_2 at ≥45% to achieve complete control (Zheng et al. 1993, Cantwell et al. 1996). However, the tolerance time of broccoli to high levels of CO_2 (30–50%) is usually shorter than the desired time interval for pest control (Cantwell et al. 1995, 1996; Mitcham et al. 2001; Zheng et al. 1993).

Oxygen levels <1% are usually referred to as ultralow. CA treatments with ultralow oxygen (ULO) levels (referred as ULO treatments hereafter) also have been studied for postharvest insect control on fresh commodities (Mitcham et al. 2001, Liu 2005). Successful control of the aphid *Nasonovia ribisnigri* (Mosley) on iceberg lettuce, *Lactuca sativa* L., was achieved using ULO treatment without injury to lettuce quality (Liu 2005). However, storage of fresh produce under low oxygen can cause injuries to the products, because fresh produce may undergo anaerobic metabolism that may produce more carbon dioxide and organic odors such as ethanol and acetaldehyde (Beaudry 2000, Mitcham et al. 2001). For broccoli, storage under low oxygen conditions may lead to production of methanethiol and other off-odor chemicals (Kasmire et al. 1974, Obenland et al. 1994, Pentima et al. 1995, Forney and Jordan 1999, Hansen et al. 2001, Toivonen and DeEll 2001, Tulio et al. 2003). ULO treatments for pest control use much lower oxygen levels than those for low oxygen storage, and they have shorter durations relative to storage (Liu 2005). The potential of such ULO treatments for postharvest control of western flower thrips has not been explored. An effective ULO treatment not only pro-

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vides an alternative to methyl bromide fumigation but also provides an option for postharvest insect control for organic produce.

In this study, western flower thrips were subjected to various ULO treatments to develop effective ULO treatments for thrips control. Commercial broccoli was subjected to selected ULO treatments to determine efficacy of the ULO treatments for thrips control and effects on postharvest quality of broccoli.

Materials and Methods

Insects and Plants. Western flower thrips were reared on lettuce plants in a greenhouse. Potted lettuce plants were placed in (10 × 1.5 cm) petri dishes on a layer of sandy soil in trays to allow thrips to pupate in the soil. Plants were watered daily. Thrips (larvae and adults) were collected in plastic vials (2.5 cm in diameter by 7 cm in height) with lettuce leaf pieces using a vacuum-powered aspirator. Each vial contained 10–20 thrips. The vials were sealed with screened lids lined with Kimwipe tissue to prevent escape of thrips.

Commercial broccoli in crowns or bundles packed in standard broccoli cartons from Tanimura & Antle Co. and Dole Fresh Vegetables (Salinas, CA) was used in this study. Most broccoli was taken from commercial coolers after being packed with ice on the day of harvest. Some broccoli was delivered directly from field without ice and was topped with ice before being used in experiments. Broccoli cultivars used in this study included 'Greenbelt,' 'Heritage,' 'Patriot,' and 'Patron.'

Responses of Western Flower Thrips to ULO Treatments. Two ULO treatment protocols were used. In the first treatment protocol, 6- and 10-d treatments with 0.023–0.03% oxygen were conducted using metal drum chambers in refrigerators at 1°C. In the second treatment protocol, 2–6-d treatments with 0.003% oxygen were conducted in plastic jars linked to a large box chamber filled with broccoli in a walk-in cooler at 1°C.

For the longer term treatments of 6 and 10 d, commercial broccoli was placed on a platform slightly raised from the bottom of the metal drum (76 liters). The broccoli crowns were laid on the platform and toped with ice. This setup prevented broccoli from being soaked in water from melted ice. By the end of 6-d treatment, there was still some ice on the broccoli. All ice melted by the end of the 10-d treatment. A 12-V d.c. fan was placed above the broccoli to circulate air constantly. Western flower thrips were collected in the plastic vials as described above. The plastic vials were placed in larger plastic vials (5 cm in diameter by 8.5 cm in height) with openings facing the bottoms of the larger vials, and the larger vials were sealed with screened lids. The larger vials were placed upside down between broccoli heads, and the smaller vials with thrips were positioned upward. This double-vial setup protected thrips from getting wet. The drum chambers were placed in refrigerators equipped with circulation fans and external temperature controllers at 1 ± 0.5°C. The metal drum chambers and the procedures to establish ULO conditions were detailed by Liu (2005). An oxygen analyzer (model 810, Illinois Instruments, Inc., Johnsburg, IL) was used to monitor oxygen levels in the treatment chambers. Oxygen levels in the chambers were reduced by flushing the chambers with nitrogen gas with ≈0.1% oxygen from a nitrogen generator (Balston 75-7820, Parker Hannifin Co., Tewksbury, MA), and the desired oxygen level for treatment was maintained by intermittent release of commercial nitrogen from compressed cylinders into the chambers. Small amount of air regulated with a flow meter was added to nitrogen gas flow when oxygen level in the treatment chambers fell below the set point of 0.03%. This addition was controlled by an alarm relay of the oxygen analyzer.

The 6- and 10-d treatments were replicated at two different times. Numbers of thrips varied among different treatments: 138 and 97 thrips in the 6- and 10-d ULO treatments and 83 and 63 thrips in the 6- and 10-d controls. After each treatment, thrips in plastic vials were held in an environmental chamber for 1 d at 24°C under a photoperiod of 14:10 (L:D) h before being scored for mortality. Insects that did not move and failed to respond to repeated probing with a soft brush were scored as dead.

In the second ULO treatment protocol, western flower thrips in plastic vials were set up in plastic jars linked to a box chamber (107 by 74 by 71 cm) containing three cartons of broccoli packed with ice to determine effects on survival of western flower thrips under 0.003% oxygen for different durations at 1 ± 0.5°C by using similar procedures as detailed by Liu (2005). A plastic vial with moist paper towels was added in each jar to maintain high humidity during the treatment. ULO conditions were established in the large box chamber. The box chamber was first flushed with generated nitrogen gas. Because much lower oxygen level was used in the treatments, a mix of commercial nitrogen gas with almost no oxygen and generated nitrogen with ≈0.1% oxygen was then released into the box chamber intermittently (10 s/5 min at a flow rate of ≈20 liters/min) to maintain desired ULO condition for the treatment.

Once a desired ULO condition was established, a series of jars with thrips in plastic vials and an air pump (SP6000, Smart Products, Inc., Morgan Hill, CA) were flushed with nitrogen and connected to the box chamber with one end of the jar series connected to one port on the box chamber and the another end of the jar series connected to a separate port on the box chamber. The ULO atmosphere in the box chamber was circulated by the air pump through the treatment jars for certain lengths of time to complete specific ULO treatments. Individual jars in a series were taken out at different times to achieve different treatment durations. This was accomplished by turning off the air pump and disconnecting a jar from a series and quickly reconnecting the tubing to allow air circulating from the box chamber through the jar series to continue the ULO treatments.

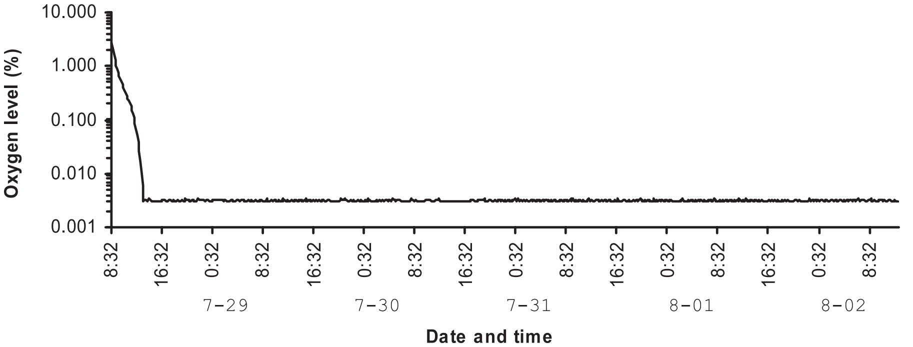

Fig. 1. Recording of oxygen levels in a 5-d ULO treatment for control of western flower thrips on broccoli at 1°C in 2006. Oxygen levels were recorded every 10 min during the treatment.

Four treatments with different durations of 2, 3, 4, and 5 d were conducted. Each treatment was replicated two to three times. In total, 581 thrips were treated, and 70 thrips were used as controls. Controls were kept in the same walk-in cooler as the treatments at 1°C for 5 d. Insect mortality was scored using the same procedures as stated above.

Effects of Large-Scale ULO Treatment on Mortality of Western Flower Thrips and Visual Quality of Broccoli. Large-scale tests were conducted in the box chambers in the walk-in cooler at 1°C to confirm ULO effectiveness in controlling western flower thrips and to determine effects of ULO treatment on postharvest quality of broccoli. Four- and 5-d ULO treatments with 0.003% oxygen were conducted. Three cartons of ice-packed broccoli were placed in each box chamber. A treatment in each box chamber was one replication. One carton of broccoli of each cultivar was held in the same cooler at 1°C as control for each treatment replication.

ULO treatment with 0.003% oxygen was initially conducted using the same procedures as stated above. Further modifications to the procedures were made for remainder of ULO treatments. The box chamber was modified by adding a trough along the rim to receive the lid. The trough was filled with water. When the box was covered with the lid, the lip of the lid extended down into the water in the trough to achieve airtight seal of the box. After ULO condition was established in the treatment chamber, commercial nitrogen from compressed cylinders was released into the box chamber continuously at 1 l/min. Generated nitrogen with 0.2% oxygen was released to the box once oxygen level in the chamber fell below set level of 0.003%. The oxygen level was recorded every 10 min and ranged between 0.00296 and 0.00340% after ULO condition was established (Fig. 1). A carbon dioxide analyzer (model 302M, Nova Analytical Systems, Inc., Niagara Falls, NY) was used to monitor carbon dioxide levels in the treatment chambers periodically, and carbon dioxide levels were below 1% through out the ULO treatments.

Both the 4-d ULO treatment and 5-d ULO treatment were replicated five times (five box chambers). Fifteen cartons of broccoli were tested in the 4-d treatments, and 15 cartons of broccoli were tested in the 5-d treatments. In the 4-d treatments, thrips were set up in double vials as described above. In total, 580 thrips were treated and 156 thrips were used as controls. In the 5-d treatments, thrips were set up in double vials for the first two tests and whole lettuce plants infested with thrips in screened plastic cups were used in the last three tests. In total, 2,767 thrips were treated, and 558 thrips were used as controls. After each test, thrips were kept in the environmental chamber for 1 d before being scored for survival as described above.

For 5-d ULO treatment, broccoli from controls and the ULO treatments were stored at 1°C in a walk-in cooler for 2 wk after ULO treatment. Postharvest quality was then evaluated. Quality parameters evaluated included color, tightness of beads, presence of dead beads, and off-odor development. Deterioration of broccoli quality is marked by change of color from dark green to yellowish, opening of beads, or off-odor development resulting from decay of individual beads. ULO-treated broccoli was evaluated with the control side by side for visual appearance. Digital photographs of treated and control broccoli were taken to record visual appearance of broccoli. The surfaces of broccoli crowns were shaved with a knife to cut across beads. This was to help to detect dead beads. Any occurrence of dead beads or off-odor development also was recorded. An expert from Tanimura & Antle Co. also participated broccoli quality evaluation for two of the 5-d ULO treatments.

For the last three ULO treatments, color of broccoli crowns was measured at the start of ULO treatment and at the time of quality evaluation using a spectrophotometer (ColorTec-PSM, Accuracy Microsensors, Inc., Pittsford, NY). For each ULO treatment, five or 10 crowns of broccoli were taken from the control and ULO treatment for color measurements. Three color measurements were taken for each broccoli crown at different positions. The average values of L^* (luminosity), a^* (green-red), b^* (blue-yellow), and $H°$ (hue angle, $H° = \tan^{-1}[b^*/a^*]$) parameters for each crown were collected for analyses.

Data Analyses. The number of thrips in each plastic vial or cup was used as basic data unit in data analyses. Insect mortality in each vial was used to calculate

Table 1. Effects of oxygen level and treatment time on mortality of western flower thrips at low temperature (1°C)

O_2 level (%)	Treatment time (d)	No. thrips	Mortality (%)[a] (mean ± SE)
Treatments in metal drum chambers			
0.030	6	138	85.7 ± 5.7
0.023	10	97	100
Treatments in plastic jars			
0.003	2	153	48.5 ± 7.3c
0.003	3	151	85.3 ± 2.6b
0.003	4	147	99.3 ± 0.7a
0.003	5	130	100a
Treatments in large box chambers			
0.003	4	580	95.9 ± 0.8
0.003	5	2,767	100

[a] Mortality was corrected for control mortality using Abbott's formula. The insect mortality rates for the two treatments in the drum chambers were significantly different based on *t*-test. In treatments in plastic jars, mortality rates followed by the same letter were not significantly different based on Turkey–Kramer multiple range test. The insect mortality rates for the two treatment times in the large box chambers also were significantly different based on *t*-test ($P = 0.05$; JMP Oneway platform, SAS Institute 2002).

mortality in treatments and controls. Abbott's method was used to adjust insect mortality rates in treatments to correct for control mortality. Mortality data were transformed by arcsine square root before statistical analysis. Within each type of ULO treatments, *t*-test was used to compare two treatment means, and one-way analyses of variance (ANOVAs) and Turkey–Kramer multiple range tests were used to compare insect mortality means for treatments in plastic jars using Oneway platform of JMP statistical software (SAS Institute 2002).

For color measurements, mean L^*, a^*, b^*, and $H°$ parameter values for each broccoli crown were analyzed using ANOVA. Tukey–Kramer multiple range test was used to compare L^*, a^*, b^*, and $H°$ values among the three categories: pretest, control, and ULO treatment (Oneway platform of JMP statistical software, SAS Institute 2002). ΔE is the total color difference and defined as $\sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$. ΔL^*, Δa^*, and Δb^* were differences in L^*, a^*, and b^* measurements between two treatments. The total color difference (ΔE) values were calculated between pretest and ULO treatment and between control and ULO treatment.

Results

Western flower thrips were controlled successfully at both oxygen levels tested with different treatment durations. The 10-d ULO treatment with 0.023% oxygen killed all thrips. In the 6-d treatment with 0.03% oxygen, thrips mortality was ≈86% (Table 1). Control mortality rates were 21.7 ± 4.1 and 47.1 ± 6.4% at 6 and 10 d, respectively. At the end of 10 d with 0.023% oxygen, treated broccoli had noticeable off-odor development at the time of treatment termination.

In ULO treatments in plastic jars at further reduced oxygen level of 0.003%, complete control of thrips was achieved in 5 d. Treatment time has significant effects on thrips mortality. Mortality increased dramatically from 48.5% at 2 d to 99% at 4 d (Table 1). For all treatment durations in plastic jars, control mortality of 14.7 ± 6.2% at 5 d was used to calculate corrected mortality.

In the larger scale ULO treatments, thrips mortality rates were 95.9% at 4 d and 100% at 5 d (Table 1). Mortality rates in controls were 31.3 ± 7.3 and 32.2 ± 7.3%, respectively. No off-odor development was noticed at the time of treatment termination. After 2-wk of post-treatment storage at 1°C, there was also no off-odor development for either the ULO treatment or the control. Side-by-side comparisons between ULO treatment and control revealed no differences in color and tightness of beads. Both ULO-treated broccoli and control broccoli had dark green color, tight beads, and no apparent visible degradation. For most broccolis from both treatment and control, no dead beads were found after shaving the surfaces of broccoli crowns. In one rare instance, a few broccoli crowns from both ULO and control were found to have one to two dead beads.

For color measurements, there were no significant differences in L^*, a^*, or b^* among pretreatment, ULO treatment, and control ($P = 0.847$, 0.153, and 0.666, respectively). However, there were significant differences in hue angle ($H°$) with the highest for pretest and lowest for ULO treatment ($P < 0.0001$) (Table 2). The total color difference ΔE between pretest and ULO treatment was 0.98. The total color difference ΔE between control and ULO treatment measured after 2 wk of posttreatment storage was 0.59 (Table 2).

Discussion

The results of this study indicated that shorter ULO treatment with lower oxygen level was more suitable than longer ULO treatment with higher oxygen level

Table 2. Effects of 5-d ULO treatment with 0.003% O_2 at 1°C on color parameters of broccoli after 2 wk of posttreatment storage

Treatment	n[a]	L^*	a^*	b^*	$H°$	ΔE[b]
Pretest	25	38.87 ± 0.28a	−5.94 ± 0.19a	10.33 ± 0.48a	120.53 ± 0.62a	0.98
Control	20	38.76 ± 0.56a	−5.77 ± 0.32a	10.89 ± 0.62a	118.31 ± 0.83b	0.59
ULO	20	38.48 ± 0.64a	−5.25 ± 0.27a	10.91 ± 0.49a	115.84 ± 0.82c	

Values in each column followed by different letters were significantly different (Tukey–Kramer multiple range test, $P \leq 0.05$; JMP Oneway platform, SAS Institute 2002).

[a] Total number of broccoli crowns from three tests.

[b] Each value was calculated using L^*, a^*, and b^* values from ULO treatment as reference.

for control of western flower thrips on broccoli. The 5-d treatment with 0.003% oxygen at 1°C caused no significant damage or loss of market quality to broccoli. The melting of ice was minimal; therefore, the treatment is compatible with ice-packed shipping of broccoli. However, the treatment is also expected to be suitable for broccoli shipped without ice.

Even though 100% mortality of thrips was achieved in 5-d treatment, complete control was also achieved in two of five replicates of the 4-d treatments, and the average mortality in 4-d treatments was >95%. There may be additional mortality during transit after ULO treatments as indicated by higher mortality of thrips in the controls stored at low temperature for 10 d versus stored for 5 or 6 d (Table 1). Therefore, the duration of ULO treatment for complete control of thrips may be further reduced. The treatment duration did not include the time needed to establish the ULO condition for the treatment, which depended on purity and flow rate of nitrogen used and amount of void space existed in the treatment enclosure. The oxygen level of 0.003% also can be modified to a certain extent without causing significant changes to thrips mortality. However, further increases in oxygen level to 0.01% or higher would need significant increases in treatment duration to achieve complete control of thrips (Y.-B.L., unpublished data).

Because of the much lower oxygen level of 0.003% and different treatment procedures used in the current study compared with other studies, comparisons of current results with those published were problematic. For the 10-d treatment with 0.023% oxygen, off-odor development at the end of the treatment was consistent with a previous report of offensive odor development after holding broccoli at 0 and 2.5°C for 10 d (Kasmire et al. 1974). Increased carbon dioxide and reduced oxygen levels correlated with strong off-odor development (Kasmire et al. 1974, Obenland et al. 1994). Off-odor development was also reported to be insensitive to carbon dioxide levels and increased with temperature (Izumi et al. 1996). The success of the present 5-d ULO treatment was probably due to the combination of ULO level, low temperature, and short treatment time. A preliminary taste survey also showed that ULO treatment had no negative impact on sensory quality of broccoli, suggesting that ULO treatment was safe to broccoli quality (Y.-B.L., unpublished data).

For the color measurements, $H°$ represents greenness. $H°$ value for fresh broccoli also varies significantly between 120 and 130 among broccoli varieties (Schonhof et al. 2004) and declines over time during storage (Ren et al. 2006). Although $H°$ for ULO treatment was significantly lower than for control and pretest, the magnitude of the decline was small. All other parameters of color were almost identical between ULO treatment and control. The ΔE values, which measure total color differences, were also very small compared with much greater variation of ΔE value during storage (Ren et al. 2006). In addition, color of broccoli from ULO treatment and control could not be differentiated by eyes, suggesting ULO treatment had negligible or no effects on broccoli color.

ULO treatment can be commercially applied in CA rooms before shipping or in CA shipping containers in-transit. Nitrogen gas would be needed to reduce oxygen level initially. Subsequently, oxygen consumption by respiration of broccoli would help to maintain a low oxygen level. Once desired ULO condition was established, either ambient air or nitrogen mixed with air would be needed to be released into the treatment rooms or containers to compensate for oxygen consumption by broccoli respiration. Because of extremely low oxygen used in the treatment and consumptions of oxygen in the respiration of broccoli, active air circulation would be needed to avoid local depletion of oxygen in the controlled atmosphere rooms or containers (Kasmire et al. 1974). For the same reason, the added air or nitrogen with certain levels of oxygen should be released at multiple positions in the treatment rooms or containers to maintain a uniform oxygen level in the space.

In summary, this study provided an effective ULO treatment for control of western flower thrips on broccoli at low storage temperature. The treatment lasted 5 d and was compatible with ice-packed shipping method of broccoli. The treatment had no negative effects on postharvest quality or shelf-life of broccoli. The ULO treatment has potential to be developed and used as an alternative to methyl bromide fumigation for control of western flower thrips on exported broccoli.

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